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BIOACCUMULATION OF POLYCHLORINATED DIBENZODIOXINS AND DIBENZOFURANS IN CATFISH AND CRABS ALONG AN ESTUARINE SALINITY AND CONTAMINATION GRADIENT

KIRK E. DEAN,*† MONICA P. SUAREZ,† HANADI S. RIFAI,‡ RANDY M. PALACHEK,† and LARRY KOENIG§
†Parsons Corporation, 8000 Centre Park Drive, Suite 200, Austin, Texas 78754-5140, USA
‡Department of Civil and Environmental Engineering, University of Houston, 4800 Calhoun, Houston, Texas 77204-4003, USA
§Texas Commission on Environmental Quality, MC-203, P.O. Box 13087, Austin, Texas 78711-3087, USA

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Abstract—Elevated but variable levels of polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDD/Fs) were observed in hardhead sea catfish (HH) and blue crabs (BCs), as well as in water and sediment, of the Houston Ship Channel system, Texas, USA. It is hypothesized that the variation was caused by the spatial variability of PCDD/F contamination, together with the natural mobility of organisms in satisfying prey, temperature, salinity, and reproductive requirements. Structural equation modeling was applied to explore the congener-specific relationships between PCDD/F levels in HH and BC tissues and independent predictors such as PCDD/F contamination levels, environmental factors such as salinity and temperature, temporal–spatial factors such as site depth and season, and biological factors such as length, weight, and lipid content. Contamination levels in both sediment and water were statistically significant predictors of the levels of less chlorinated congeners in both HH and BCs, with the standardized regression weight for sediment concentration roughly twice that for the water concentration. This implies that sediments are the dominant route for PCDD/F exposure and remediation efforts should focus on legacy sediment contamination. Tissue lipid content was a significant predictor of tissue concentrations in HH but only to a lesser extent in BCs, perhaps due to their low lipid content. Site depth and seasonal factors also were significant predictors of tissue concentrations. For the highly chlorinated congeners, only a small fraction of the variance in tissue concentrations was explained by the independent predictors, possibly indicating that uptake and elimination kinetics, biotransformation processes, or both may be more important factors controlling the bioaccumulation of those congeners.

Keywords—Dioxin Bioaccumulation Structural equation modeling

INTRODUCTION

Elevated levels of 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in hardhead sea catfish (*Ariopsis felis* Linnaeus; HH) and blue crabs (*Callinectes sapidus* Rathbun; BCs) of the Houston Ship Channel (HSC; Houston, TX, USA) system were first observed by the Texas Department of Health in 1990. Seafood consumption advisories were issued for these species at that time. Elevated PCDD/F levels have also been observed in water and sediments of the system [1]. Although the sources of contamination are believed to be primarily historical, contaminated sediments continue to serve as an internal source of PCDD/Fs to the water bodies, supplemented by other continuing sources including atmospheric deposition, runoff, and industrial and domestic wastewaters [1,2]. The levels of PCDD/Fs in fish, particularly catfish, and BCs have not been observed to decline since they were first quantified in 1990 [1]. A better understanding of the bioaccumulation behavior of PCDD/Fs in fish and crabs in the HSC may provide more accurate estimates of loading reductions necessary to achieve safe thresholds for seafood consumption.

Theory

Bioaccumulation involves the uptake of chemicals by aquatic organisms from their environment via multimedia exposures. It can include direct uptake from the aqueous dissolved phase, via respiratory exchange or dermal transfer, and dietary exposure with gastrointestinal uptake. In the equilibrium partitioning (EqP) model [3], the bioaccumulation of PCDD/Fs and other nonpolar hydrophobic organic compounds by aquatic organisms is commonly considered to be linearly related to the lipid content of the tissue (which indicates the sorptive capacity of the tissue), the fugacity of the contaminant in the system in which the organism is exposed, and the hydrophobicity of the contaminant (usually indicated by an octanol–water partition coefficient). The fugacity, or partial pressure, of the contaminant is the concentration in an environmental medium divided by the fugacity capacity of that medium [4]. At thermodynamic equilibrium, the fugacities in various phases are equal. The soot or organic carbon content is commonly considered to control the fugacity capacity of sediments [5,6], while lipid content is considered to control the fugacity capacity of most tissues [7]. In water, suspended solids and dissolved and colloidal organic carbon content may increase the fugacity capacity [8]. Given efficient dietary uptake and minimal biotransformation, biomagnification of contaminants in the tissues of organisms can occur when tissue fugacities exceed those in the surrounding environment (water and sediments).

The bioaccumulation factor (BAF) has been defined by the U.S. Environmental Protection Agency (U.S. EPA) as the ratio (in liters per kilogram of tissue) of the concentration of a chemical in the tissue of an aquatic organism to its concentration in water in situations where both the organism and its food are exposed and the ratio does not change substantially over time [9]. Recognizing the importance of

* To whom correspondence may be addressed (kirk.dean@parsons.com).
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tissue lipid content on the fugacity capacity of tissue, and that of suspended solids and dissolved and colloidal organic content on the apparent fugacity capacity of water, the U.S. EPA also defined a baseline BAF as a BAF that is based on the concentration of chemical freely dissolved in water and the concentration of the chemical in the lipid fraction of tissue. The BAF is an equilibrium concept and accounts for uptake and elimination from all sources and processes, including uptake via the diet and across the gill surface. Similarly, a biota–sediment accumulation factor (BSAF) has been defined as the ratio (in kilograms of sediment organic carbon per kilogram of lipid) of the lipid-normalized concentration of a chemical in tissue of an aquatic organism to its organic carbon-normalized concentration in surface sediment in situations where the ratio does not change substantially over time, both the organism and its food are exposed, and the surface sediment is representative of average surface sediment in the vicinity of the organism.

Although the EqP model is useful in predicting the potential bioaccumulation of contaminants, many studies [10–17] have shown that it sometimes fails to accurately predict observed BAFs and BSAFs. Of particular concern with PCDD/Fs, large and extremely hydrophobic compounds may not achieve the levels of bioaccumulation predicted by the EqP model. Various explanations have been offered for these observations. Chemical fugacities in sediments may not be in equilibrium with those in the overlying water column, resulting in variable bioaccumulation depending on the major route of exposure of an organism (sediment versus water) and that of its prey [10]. In addition, the EqP model does not account for variations in the composition of tissue lipids [18] and sediment organic matter [11,19] that affect bioavailability or fugacity capacity of these phases. The permeation of large molecules through cell membranes may be substantially hindered due to steric factors [11–13,20], thus limiting their bioaccumulation. Low dietary uptake efficiencies [14,15] and rapid elimination via feces [15,21] and biotransformation [10,15,16] may also limit the bioaccumulation for these compounds. Finally, the EqP model does not explicitly account for changes in an organisms' diet [11], reproductive status, nutritional status, growth [21,22], or mobility [17], to the extent that they occur at a rate faster than the compounds are taken up or eliminated from tissue.

The relative impact of water and sediments in determining bioaccumulation is of major interest to evaluating the effectiveness of various contamination remediation strategies. If the majority of contaminant bioaccumulation occurs via a sediment route, the remediation of legacy sediment contamination may be necessary. On the other hand, if contaminant bioaccumulation occurs via a waterborne route, it may be more effective to achieve further reductions of ongoing discharges and deposition of PCDD/Fs to water.

Study area

The HSC is a dredged channel 13.7 m deep and 162 m wide extending approximately 86 km from the Gulf of Mexico at Galveston to near downtown Houston. The lower 46 km of the HSC extend unconfined from the Gulf of Mexico through Galveston Bay, a large (1,317 km^2), shallow (2.1 m average depth) embayment. The middle 15 km of the HSC from Morgan's Point up to the confluence with the San Jacinto River are partially confined, but numerous small, shallow embayments abut and connect to the HSC. Upstream of the confluence with the San Jacinto River, the HSC runs 25 km westward along Buffalo Bayou in a confined channel with several tributary bayous.

The HSC–Galveston Bay system is tidally influenced, and freshwater inflow exerts a strong influence on salinity levels. In most parts of Galveston Bay, salinities average 15‰, indicating approximately equal contributions of freshwater and saltwater. The salinities decline with distance upstream, and average salinities are approximately 6‰ in the upper reaches of the HSC. In tidal tributaries to the upper reach of the HSC, salinities are often less than 1‰. Freshets occur throughout the year, associated with major rainfall events, but inflow is typically highest in April and May and lowest from July through October. A moderate vertical salinity gradient typically occurs in the deep channels, with denser saltwater inflows along the bottom and freshwater inflows on top. However, vertical mixing tends to be strong in the system and the shallow bays are seldom stratified.

Contamination due to PCDD/Fs is not uniform throughout the HSC system. Two areas in the upper reaches of the HSC system are particularly contaminated with PCDD/Fs [1,2]. An industrial waste pit was operated in the 1960s and 1970s along the banks of the San Jacinto River near Interstate Highway 10. With land subsidence of several feet in recent decades, the waste pit was submerged in the San Jacinto River, likely causing substantial PCDD/F contamination throughout the HSC system. The level of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exceeded 20,000 ng/kg dry weight in one sediment sample from this site. A more diffuse area of elevated PCDD/F levels in sediment occurs in the Buffalo Bayou portion of the HSC upstream of the confluence with the San Jacinto River, with TCDD levels as high as 650 ng/kg in sediments. Levels of PCDD/Fs tend to decline with distance upstream and downstream from these two most contaminated areas, and PCDD/F levels in the water and sediment of upper Galveston Bay are much lower than in the more contaminated areas, with TCDD levels seldom exceeding 1 ng/kg in sediment. Concentrations of PCDD/Fs in water exhibit less spatial variation than those in sediment. Median levels of TCDD in water were 1.1 pg/L near the most contaminated site in the San Jacinto River at Interstate Highway 10, 0.23 pg/L in the more contaminated areas of the HSC upstream of the San Jacinto River, and 0.05 pg/L in upper Galveston Bay.

Both BCs and HH live and feed primarily along the sediment surface and are opportunistic omnivores and scavengers that occupy a similar midtrophic level niche. The diet of HH in the HSC system is dominated by shrimp, crabs, stomatopods, organic detritus, and small fish [23]. The diet of BCs includes mollusks, shrimp, crabs, small fish, plants, and organic detritus [24].

Both BCs and HH are sensitive to the temperature and salinity variations in a subtropical estuary. During winter months, when average water temperatures fall below 15°C, HH are rare in the HSC system and are believed to migrate into the deeper offshore waters of the Gulf of Mexico [25]. Large volumes of freshwater inflow may also cause HH to migrate periodically from the upper and middle reaches of the HSC to the lower reaches of Galveston Bay and the Gulf of Mexico. McElyea [26] reported a strong positive correlation between HH abundance and salinity in the upper HSC.

Adult male BCs tend to prefer the low-salinity waters of the upper estuary and are the dominant sex in most of the HSC system, while females prefer salinities above 20‰ and migrate to the Gulf of Mexico to spawn [24]. The abundance of BC in

the upper HSC was observed to be positively correlated with temperature [26], and crabs may migrate to the deeper waters of the Gulf of Mexico or burrow into mud and enter a state of torpor during the winter [27].

The spatial variability of PCDD/F contamination in the HSC system, together with the mobility of HH and BCs (due to food availability, life cycles, and their environmental preferences for temperature and salinity conditions), results in spatially and temporally variable exposures to PCDD/Fs. For risk assessment purposes, the seasonal and spatial variation in bioaccumulation was of particular interest. Temporally and spatially uniform tissue fugacities (lipid-normalized concentrations) of PCDD/Fs would imply that the processes of biouptake and elimination were slow relative to the variations in PCDD/F exposures. On the other hand, relatively constant BAFs and BSAFs would indicate that these species achieved rapid equilibrium with the exposure conditions at the site and time they were sampled.

MATERIALS AND METHODS

At each site, approximately 700 L of water were pumped at a rate of 1.6 L/min through an Infiltrex 300 high-volume water sampling system (Axys Technologies). Water was first passed through a wound glass fiber filter cartridge (10 cm long × 6.4 cm diameter) with a 1-μm effective pore size in order to trap particle-associated PCDD/Fs. The filtrate was then passed through a stainless steel column packed with approximately 250 g of Amberlite® XAD-2 hydrophobic cross-linked polystyrene resin (Rohm and Haas) to trap dissolved PCDD/Fs. Experiments with two XAD-2 resin columns in series showed that they trapped dissolved PCDD/F efficiently, with little or no breakthrough to the second column. The PCDD/Fs associated with colloids such as humic substances were considered to pass through the XAD-2 columns at the neutral–high ambient pH of the HSC. Other investigators using similar hydrophobic resin columns observed that more than 90% of the dissolved and colloidal organic carbon passed through the columns [12,28]. However, the size of the colloidal pool of PCDD/Fs was not measured, nor was its passage through the XAD-2 cartridge verified. Simultaneously with PCDD/F sampling, grab samples were collected for measurement of total suspended solids and total and dissolved organic carbon. Temperature, salinity, specific conductance, pH, and dissolved oxygen were measured with a YSI 600XLM sonde.

Surface (0–5 cm) sediment samples were collected with a stainless steel Ponar dredge. At each site, a minimum of three grab samples were deposited in a stainless steel bowl and mixed thoroughly with a stainless steel spoon. Composite subsamples were then deposited into a labeled, precleaned amber glass jar with a Teflon®-lined lid and stored at less than 4°C until analysis.

Biota samples

We collected HH and BCs from 45 sites throughout the HSC system during the spring, summer, and fall seasons from 2002 to 2004. Site locations are shown in Suarez et al. [2]. A total of 108 paired HH, sediment, and water samples and 155 paired BC, sediment, and water samples were collected from the 45 sites.

Hardhead catfish were collected using gill nets, fish traps, or hook and line. All specimens were adults exceeding 28 cm in length. Blue crabs were collected using baited crab traps, and with few exceptions, all specimens exceeded the legal minimum carapace width of 12.7 cm. Specimens were weighed and measured, and then HH were filleted to extract muscle portions, and edible meat portions of BC were extracted. The selected tissues from each specimen were then composited and homogenized with two to four other specimens of the same species collected from the same site on the same date. These were stored frozen until analysis.

An effort was made to collect the BC, HH, sediment, and water samples from a given site on the same day. However, this proved difficult in practice and the samples from various media for a given station were collected as much as two weeks apart.

Chemical analyses

The levels of the 17 2,3,7,8-substituted tetra- through octachlorinated PCDDs and PCDFs in water, sediment, and tissue samples were quantified by high-resolution gas chromatography or high-resolution mass spectrometry using U.S. EPA method 1613 revision B (www.epa.gov/waterscience/methods/method/dioxins/1613.pdf) at a U.S. EPA–certified commercial laboratory. Tissue lipid content was determined gravimetrically by U.S. EPA method 1613B. Sediment organic carbon content was determined in a carbon, hydrogen, and nitrogen elemental analyzer following acidification for removal of inorganic carbon. Further details on analytical methods and quality control are provided in Suarez et al. [1,2].

Statistical analyses

Bioaccumulation factors and BSAFs were calculated for HH and BCs for each of the 17 congeners quantified for paired samples where PCDD/F concentrations were quantifiable in both the tissue and the sediment (for BSAFs) or in the water-dissolved phase (for BAFs). Both BAFs and BSAFs were calculated using lipid-normalized tissue concentrations.

Structural equation modeling (SEM), a multivariate statistical technique similar to multiple regression that describes a network of complex linear relationships among variables [29], was applied to ascertain the factors influencing bioaccumulation. Several potential influential factors related to PCDD/F concentrations in tissue (on a wet weight basis) were considered in exploratory SEM: PCDD/F concentrations in sediment and water (total, dissolved, and suspended), characteristics of the organisms (length, weight, length to weight ratio, and lipid content), water characteristics (temperature, salinity, pH, dissolved organic carbon, and suspended solids concentration), sediment organic carbon content, spatial variables (site depth and distance from various points), and temporal variables (year, season, month, Julian day number [1–365], and air temperature). The sampling site depth primarily distinguishes bay sites from the much deeper channel sites. Factor analysis was used to extract from the spatial variables two major factors, one indicating the distance along the channel from downtown Houston toward the Gulf of Mexico and a second indicating the distance along the San Jacinto River channel. However, it was noted that the spatial factors strongly covaried with contaminant concentrations; thus, they were not used in further analyses. The air temperature used was the average air temperature in Houston from 1970 to 2000 for each Julian day number, which peaks in the summer and then declines, whereas the Julian day number increases throughout the year.

As preliminary results indicated that models attempting to apply to all congeners and both HH and BCs neither fit well nor explained much of the observed variation, separate SEMs were developed for each species, and for each congener with more

Table 1. Summary of measured 2,3,7,8-substituted polychlorinated dibenzodioxins and polychlorinated dibenzofurans congener concentrations in various media[ab]

	Blue crab muscle, pg/g (n = 155)			Hardhead catfish fillets, pg/g (n = 108)			Dissolved concentration, pg/L (n = 148)			Sediment concentration, pg/g (n = 174)		
Congener	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.
2378-TCDD	<0.18	2.2	12	<0.36	5.15	26	<0.003	0.038	0.441	<0.23	6.45	650
12378-PeCDD	<0.10	<0.29	1.5	<0.10	0.36	4.5	<0.003	<0.007	0.028	<0.33	<0.85	13
123478-HxCDD	<0.09	<0.21	1.4	<0.10	<0.24	6.8	<0.003	0.009	0.035	<0.12	1.85	12
123678-HxCDD	<0.11	0.27	3.1	<0.11	0.64	7.6	<0.004	0.014	0.199	<0.29	3.95	71
123789-HxCDD	<0.10	<0.23	1.7	<0.11	0.29	7.5	<0.004	0.018	0.082	<0.48	3.60	24
1234678-HpCDD	<0.23	0.74	4.2	<0.41	1.15	10	0.030	0.416	4.27	1.90	120	2,100
12346789-OCDD	0.35	3.4	62	0.59	2.8	20	0.227	8.27	80.7	39	3,100	41,000
2378-TCDF	<0.21	3.8	30	<0.12	0.44	4.6	0.009	0.131	1.22	<0.16	18.5	1,600
12378-PeCDF	<0.10	<0.28	1.7	<0.07	<0.24	5.0	<0.003	0.010	0.199	<0.2	1.05	170
23478-PeCDF	<0.09	0.35	1.7	<0.12	0.54	4.4	<0.004	0.012	0.118	<0.23	1.80	180
123478-HxCDF	<0.09	<0.23	2.0	<0.07	<0.19	6.5	<0.002	0.012	0.242	<0.17	2.10	370
123678-HxCDF	<0.08	<0.20	1.6	<0.07	<0.22	7.8	<0.002	<0.009	0.257	<0.23	1.40	110
234678-HxCDF	<0.07	<0.21	1.6	<0.03	<0.21	7.5	<0.003	0.007	0.114	<0.19	1.30	51
123789-HxCDF	<0.07	<0.21	1.9	<0.03	<0.26	6.4	<0.001	<0.005	0.048	<0.20	0.67	88
1234678-HpCDF	<0.12	<0.39	14	<0.05	<0.36	9.0	<0.003	<0.054	2.21	<0.19	16.0	1,500
1234789-HpCDF	<0.10	<0.27	1.5	<0.04	<0.29	10	<0.002	<0.009	0.138	<0.25	1.70	160
12346789-OCDF	<0.21	1.0	410	<0.26	0.82	72	<0.018	0.185	38.5	<2.3	110	42,000
Tissue lipid content (%)	0.1	0.8	1.6	0.2	1.8	4.0						
Sediment organic carbon (%)										0.1	1.3	5.4

[a] n = number of samples analyzed. Each sample was composed of three to five individuals.
[b] Max. = maximum; Min. = minimum; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran.

than 40 measured values above the analytical detection limit in each of the tissue, sediment, and dissolved phases: TCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin, octachlorodibenzo-*p*-dioxin (OCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,4,7,8,-pentachlorodibenzofuran, and octachlorodibenzofuran (OCDF).

Structural equation modeling with maximum likelihood estimation was performed using AMOS 6.0 software (SPSS). The relative quality of model fit was judged by the chi-square statistic relative to the degrees of freedom, the squared multiple correlation between the dependent variable and the independent predictors, the root-mean square error of approximation, and the Akaike Information Criteria [30]. Except as otherwise noted, explanatory variables were retained in the model only if their regression weight was significantly different from zero with 95% confidence. Similarly, covariance among explanatory variables was generally retained in a model only if it was statistically significant ($\alpha < 0.05$). Structural equation models were rejected if they could be statistically rejected at $\alpha < 0.05$ based on the chi-square statistic and degrees of freedom. Other statistical analyses were performed using SPLUS software (Insightful).

RESULTS AND DISCUSSION

Concentrations of the 2,3,7,8-substituted PCDD/Fs in HH fillets ranged from less than 0.03 to 72 ng/kg and from less than 0.07 to 410 ng/kg in BC tissue (Table 1). Levels of the tetra-chlorinated congeners TCDD and TCDF were typically among the highest of the PCDD/F congeners in tissues. However, tissue concentrations of OCDD and OCDF often exceeded the levels of TCDD and TCDF. The median lipid content of BC and HH tissues was 0.8 and 1.4%, respectively.

Levels of TCDD and TCDF in HH and BC tissue appeared to be related to concentrations in sediment organic carbon and water sampled from the same site, implying that the tissues were at least partially in equilibrium with the levels in sediment and water. However, concentrations of the more chlorinated PCDD/F congeners in tissue were only weakly related to levels in sediment and water, if at all.

Tissue concentrations were significantly related to lipid levels in most cases, but the relationship was usually weaker than expected from thermodynamics-based EqP theory. This was particularly true for the more chlorinated congeners and for BC. The routine practice of lipid normalization for calculating bioaccumulation has been found by others studying animals with low lipid content to be inappropriate [31]. Stowe et al. [32] observed that lipids were only a modest predictor of polychlorinated biphenyl concentrations in Lake Michigan salmonids at the individual organism level, and Bonn [33] observed that lipid and sediment organic carbon normalizations did not reduce variance in PCDD/F fish tissue–sediment relationships.

Median lipid-normalized baseline log BAFs ranged from 4.41 to 6.68 L/kg in HH and from 4.91 to 7.03 L/kg in BCs (Fig. 1). Median log BSAFs ranged from −3.19 to −0.41 in HH and from −2.57 to −0.24 in BCs (Fig. 2). With the exception of TCDF and possibly 1,2,3,7,8,9-hexachlorodibenzofuran, the magnitude and congener patterns of bioaccumulation in BCs were similar to those for HH, likely an indication of the importance of chemical properties in controlling bioaccumulation. However, bioaccumulation of TCDF was substantially lower in HH than in BCs, and this pattern was even more accentuated with lipid normalization. A substantially reduced bioaccumulation of TCDF, relative to that of TCDD and other PCDF congeners, was also noted by Sijm et al. [34] in goldfish. They calculated a half-life for TCDF of 3.1 d in control fish but more than 7 d in fish exposed to an inhibitor of PCDD/F biotransformation, implying that biotransformation of TCDF is responsible for the reduced bioaccumulation. Loonen et al. [35] also noted rapid elimination of TCDF from guppies, with a half-life of 2.4 d compared

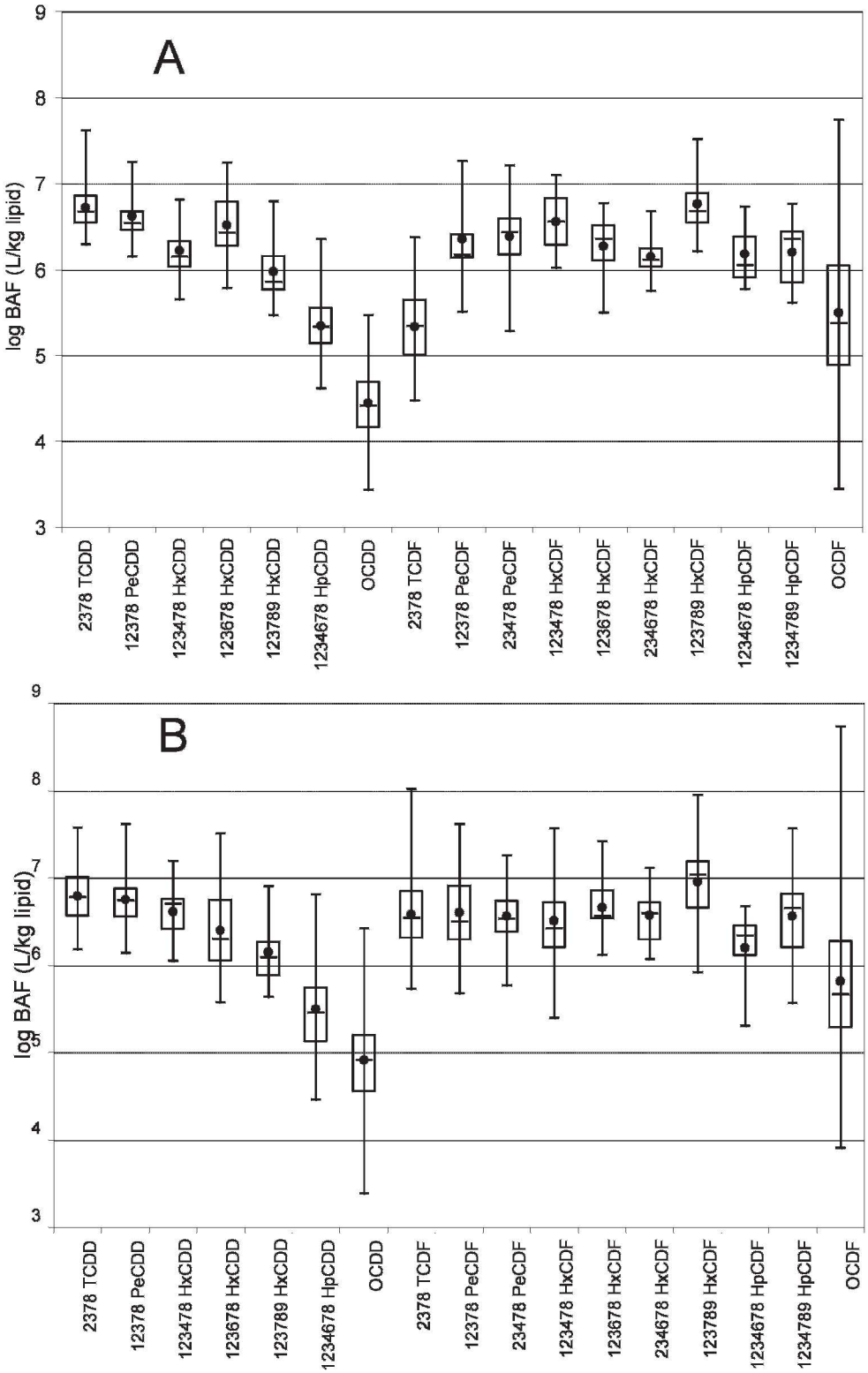


Fig. 1. Bioaccumulation factors (BAFs) of polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners in hardhead catfish (**A**) and blue crab (**B**). The first and third quartiles are represented by the vertical extent of each box, the mean by a black dot, the median by a horizontal line inside each box, and the minimum and maximum by the extent of the vertical lines extending above and below each box. Congener names are abbreviated for tetrachlorodibenzo-*p*-dioxin (TCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), octachlorodibenzo-*p*-dioxin (OCDD), tetrachlorodibenzofuran (TCDF), pentachlorodibenzofuran (PeCDF), hexachlorodibenzofuran (HxCDF), heptachlorodibenzofuran (HpCDF), and octachlorodibenzofuran (OCDF).

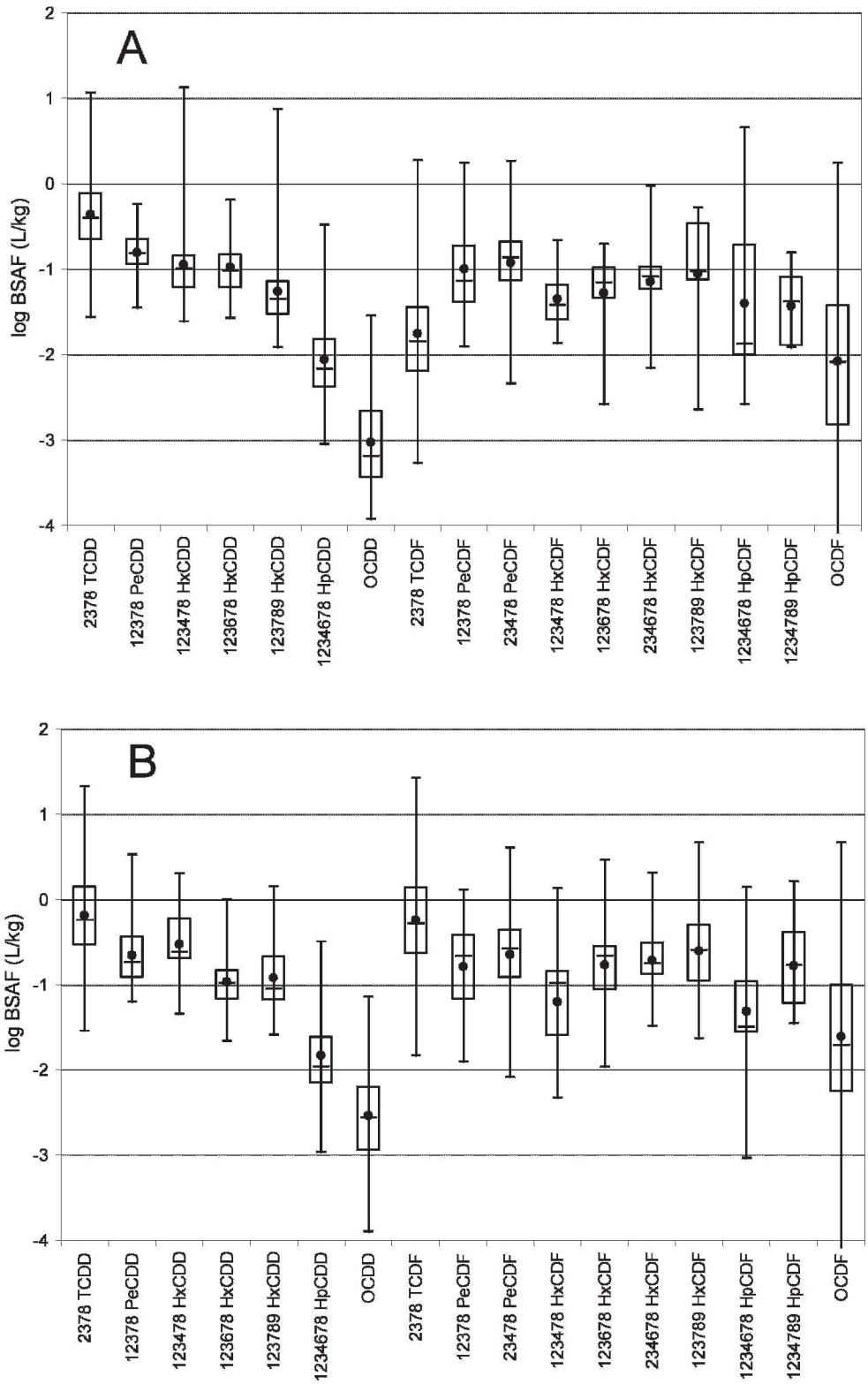


Fig. 2. Biota–sediment accumulation factors (BSAFs) of polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners in hardhead catfish (**A**) and blue crab (**B**). Congener names are abbreviated for tetrachlorodibenzo-*p*-dioxin (TCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), octachlorodibenzo-*p*-dioxin (OCDD), tetrachlorodibenzofuran (TCDF), pentachlorodibenzofuran (PeCDF), hexachlorodibenzofuran (HxCDF), heptachlorodibenzofuran (HpCDF), and octachlorodibenzofuran (OCDF).

to 14 d for TCDD. Bignert et al. [36] noted large spatial differences in the bioaccumulation of TCDF, relative to other congeners, by herring in Bothnian Bay.

For the PCDD congeners, a systematic decline in BAF and BSAF with increasing degree of chlorination, molecular size, and octanol–water partition coefficient (Fig. 3A) was noted, as has been reported by previous investigators [17,37]. This pattern is consistent with the hypothesis that permeation of cell membranes is sterically limited for larger PCDDs [11–13,20]. Low dietary uptake efficiency [14–15,38] may also limit

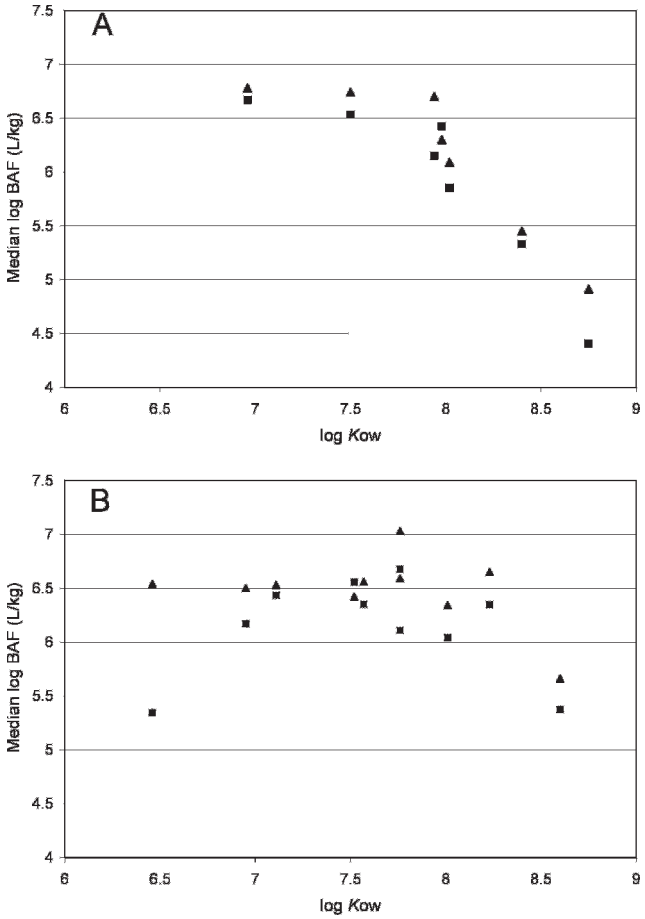


Fig. 3. Relationships between bioaccumulation factors (BAFs) of polychlorinated dibenzo-*p*-dioxins (**A**) and polychlorinated dibenzofurans (**B**) and their octanol–water partition coefficients (K_{OW}) in hardhead catfish (■) and blue crabs (▲). Values of K_{OW} are from Govers and Krop [40].

uptake of these high molecular weight congeners. Finally, given that the more chlorinated congeners also tend to be less water soluble and more prone to being strongly sorbed to sediments, alternative hypotheses involving reduced bioavailability [10] may also explain the observations.

The pattern of declining bioaccumulation with degree of chlorination was not evident to the same extent for PCDF congeners (Fig. 3B). In fact, the average BAFs and BSAFs for TCDF in HH were among the lowest of any PCDD/F congener. The absence of an observed reduction in bioaccumulation with increasing molecular size for the PCDF congeners (except for OCDF), and the apparent reduction in bioaccumulation for TCDF in HH relative to BCs, appears more consistent with a hypothesis that metabolism limits bioaccumulation of PCDFs. Burkhard et al. [10] have shown that metabolism and reduced assimilation efficiencies of PCDD/Fs reduce their BSAFs in lake trout by up to four orders of magnitude below those of relatively unmetabolizable polychlorinated biphenyls of comparable hydrophobicity. However, it should be noted that some other studies [17,37] have observed a systematic reduction in bioaccumulation with molecular size for the PCDFs congeners.

At less contaminated sites, observed BAFs and BSAFs were typically higher than those from sites where PCDD/F concentrations were higher (as illustrated in Fig. 4 for TCDD). This has been observed elsewhere [39] and may be explained as

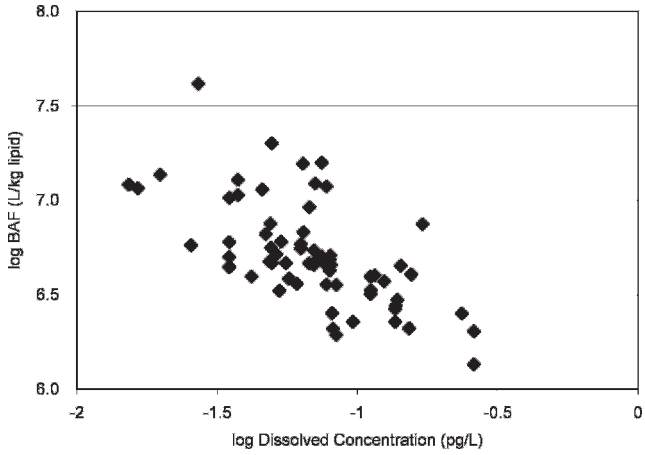


Fig. 4. Relationship between bioaccumulation factors (BAFs) in hardhead catfish and dissolved concentrations for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

follows: HH, BCs, and some of their prey are mobile organisms, and water concentrations are dynamic, so they are exposed to PCDD/F levels from more and less contaminated sites that do not represent only the site and time where they are collected. Thus, it is important to evaluate the risks of contaminant bioaccumulation in light of the temporal and spatial variability of exposure, as well as the mobility and life history of the species.

Structural equation modeling

Hardhead sea catfish. Figure 5A shows a rather complex network of factors affecting the levels of TCDD in HH tissue. This SEM explained 62% of the variance in measured concentrations, with the balance attributed to other factors not in the model (including measurement error). The tissue concentration showed a strong positive association with lipid content, with a standardized regression coefficient of 0.47. A strong association was also seen between tissue and sediment TCDD concentrations (standardized regression coefficient = 0.35). Sediment concentrations exerted more than twice as much influence on tissue concentrations as did dissolved concentrations (standardized regression coefficient = 0.15). As expected, strong covariation (0.63) occurred between sediment and dissolved concentrations. Other statistically significant factors related to tissue levels were the site depth and the Julian day number of the year. The relationship with depth primarily reflected that TCDD tissue levels of HH collected in the deep channels tended to be higher than those collected in the bays, even after accounting for the covariation between dissolved concentrations and depth (no significant independent covariation occurred between depth and sediment concentration). The covariance between dissolved TCDD concentrations and site depth likely reflected that many of the shallower Galveston Bay sites were less contaminated than those in the more confined HSC upstream. A weak but statistically significant relationship between TCDD concentrations in tissue and Julian day number was observed, which implied that tissue TCDD levels declined as the year progressed. Given that HH are known to migrate from the HSC system to presumably less contaminated waters over the winter, this result is counterintuitive. The possibility that this observation could be explained by autumn recruitment of less-contaminated juveniles into the size ranges collected was considered. However, fish length and

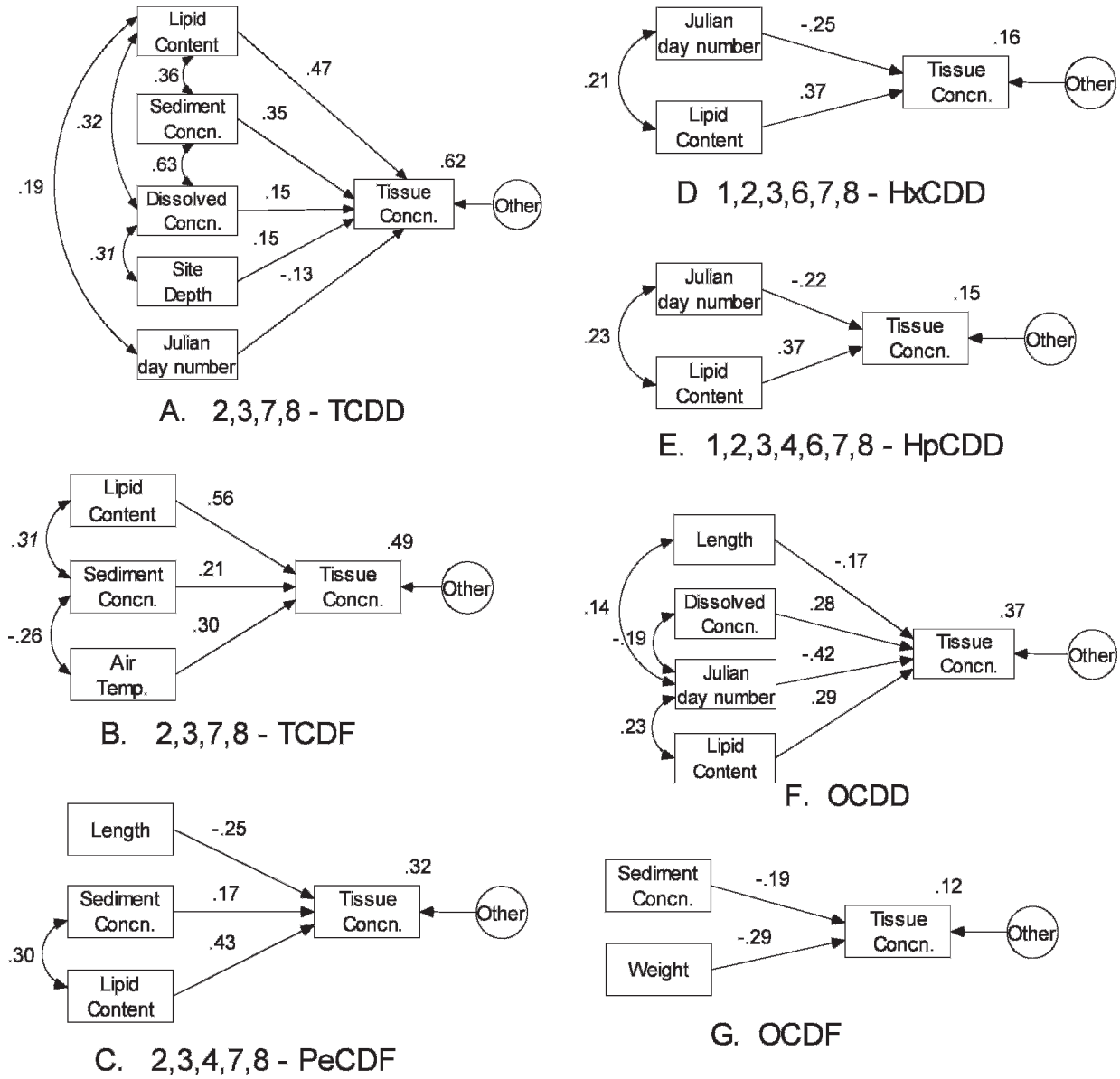


Fig. 5. Structural equation models of bioaccumulation of selected polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) congeners in hardhead catfish fillets (tissue). Double-headed arrows represent standardized covariances. Single-headed arrows represent standardized model regression weights. The squared multiple correlation (just above and to the right of the tissue box) is the fraction of the variance in tissue concentrations explained by the model. (**A**) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); (**B**) 2,3,7,8-tetrachlorodibenzofuran (TCDF); (**C**) 2,3,4,7,8-pentachlorodibenzofuran (PeCDF); (**D**) 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD); (**E**) 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD); (**F**) octachlorodibenzo-*p*-dioxin (OCDD); (**G**) octachlorodibenzofuran (OCDF).

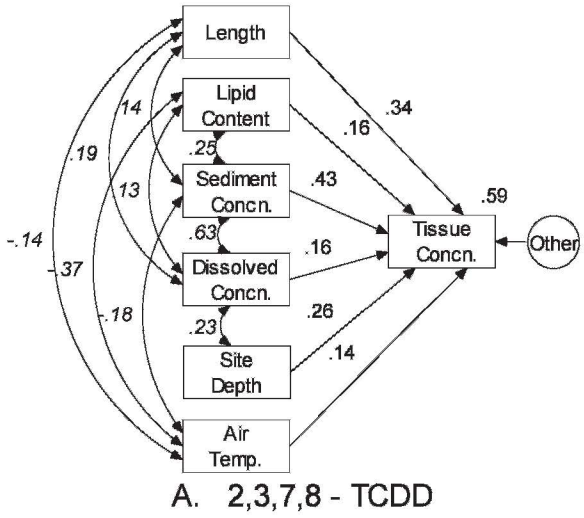
lipid content were positively related to Julian day number, indicating that HH became longer and fatter over the course of the year. Thus, it may be that the association between tissue concentration and day number resulted from growth dilution [22] or a shift to less contaminated prey. Note that lipid levels covaried with sediment and dissolved concentrations, which seems to indicate that fatter fish live in the more contaminated areas, which are also probably in the more productive, less salty waters.

It is important to note that while other explanatory variables do not appear in the SEM, this indicates not a lack of covariance with tissue concentrations but rather a covariation with the tissue concentrations that was not statistically significant after accounting for their covariation with other independent predictors in the model. Thus, it was rare for more than one strongly covarying factor (e.g., day number and air temperature or length and weight) to show up as significant in the SEM.

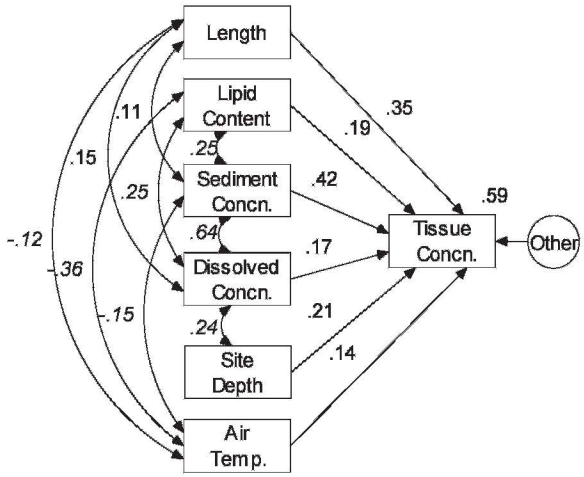
The SEM illustrated in Figure 5B explained 49% of the variability in TCDF concentrations in HH. Lipid content appeared to exert the greatest influence on observed variations in tissue levels. Other significant relationships were observed with sediment concentrations and air temperature, an indicator of seasonality.

The SEM for 2,3,4,7,8-pentachlorodibenzofuran in HH (Fig. 5C) explained only 32% of observed variability in tissue concentrations. As with TCDD and TCDF, lipid content and sediment concentrations were significant predictors of levels in tissue. The SEM also indicated that organism size in HH, as indicated by length, was inversely related to tissue levels.

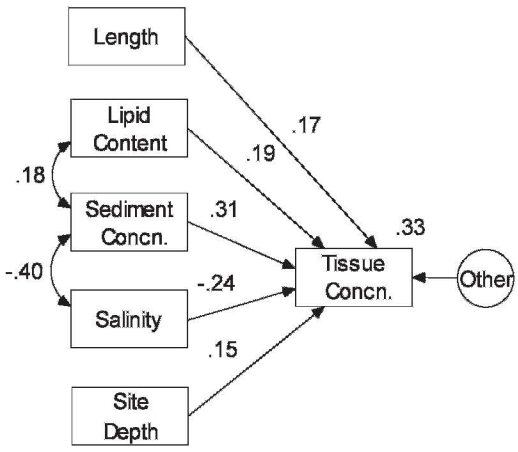
The SEMs for 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (Fig. 5D) and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (Fig. 5E)



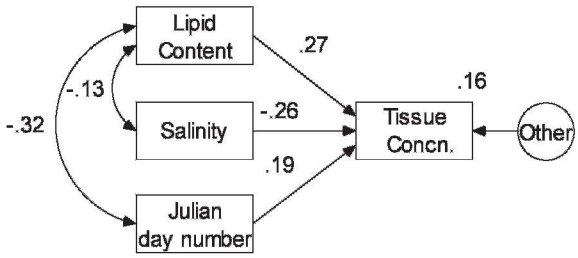
A. 2,3,7,8 - TCDD



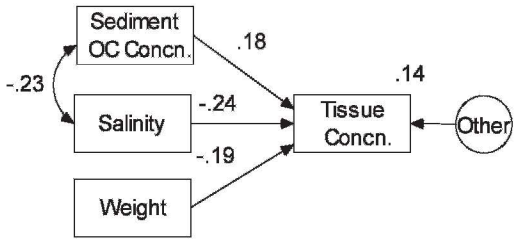
B. 2,3,7,8 - TCDF



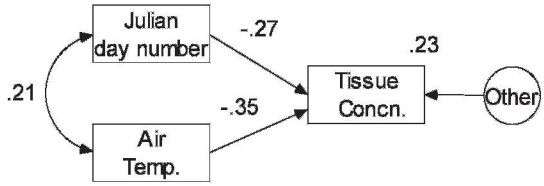
C. 2,3,4,7,8 - PeCDF



D. 1,2,3,6,7,8 - HxCDD



E. 1,2,3,4,6,7,8 - HpCDD



F. OCDD

Fig. 6. Structural equation models of bioaccumulation of selected polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) congeners in blue crab tissue. Double-headed arrows represent standardized covariances. Single-headed arrows represent standardized model regression weights. The squared multiple correlation (just above and to the right of the tissue box) is the fraction of the variance in tissue concentrations explained by the model. (**A**) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); (**B**) 2,3,7,8-tetrachlorodibenzofuran (TCDF); (**C**) 2,3,4,7,8-pentachlorodibenzofuran (PeCDF); (**D**) 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD); (**E**) 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD); (**F**) octachlorodibenzo-*p*-dioxin (OCDD).

in HH were simple and explained less than 20% of the variance in the tissue concentrations. Only lipid content and Julian day number were significantly related to HH tissue concentrations. The limited ability of these SEMs to explain variations in tissue concentrations of pentachlorodibenzofuran indicates that other factors, such as metabolism, controlled the levels in tissues.

The SEM for OCDD (Fig. 5F) explained 37% of the observed variance in tissue concentrations in HH. As with all other PCDDs in HH, lipid content and day number were significantly related to tissue concentrations. Day number was the strongest predictor of tissue concentrations, with a standardized regression coefficient of −0.42. The dissolved OCDD concentration, but not the concentration in sediment, was also significantly related to tissue concentration in this model. Finally, fish length was inversely related to tissue concentration, which may provide some support for the hypothesized impact of growth dilution or a shift to less contaminated prey species.

The SEM for OCDF in HH (Fig. 5G) explained only 12% of the variability in observed tissue concentrations. In addition to sediment concentrations, fish weight was a significant (inverse) predictor of tissue concentrations.

Blue crab. The SEM for TCDD in BCs (Fig. 6A) explained 59% of the variability in observed tissue concentrations. As seen in HH, both sediment and dissolved phase TCDD concentrations were significantly related to tissue concentrations, with sediments exhibiting the much stronger relationship by a factor greater than two. Also similar to HH, BC tissue concentrations were significantly related to the total water depth at the sampling site and lipid content. In contrast to HH, tissue concentrations of TCDD in BC were only weakly related to lipid content. The length or, more accurately, width of the BC carapace was significantly related to tissue levels, with larger BC having higher levels of TCDD.

The SEM for TCDF in BC also explained 59% of the variability in tissue concentrations (Fig. 6B). This SEM exhibited pronounced similarity to that for TCDD, implying that the processes controlling bioaccumulation are similar. However, note that this SEM for BC was very different from that for HH. The SEM for 2,3,4,7,8-pentachlorodibenzofuran in BC (Fig. 6C) was also similar to those for TCDD and TCDF but explained only 33% of the variance in tissue concentrations. Air temperature and dissolved phase concentrations were not significant predictors of tissue concentrations, but salinity emerged as a significant inverse predictor.

The SEMs for 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin in BC tissue (Fig. 6D and E) account for less than 20% of the observed variance in tissue concentrations. Salinity was expected to be inversely related to tissue concentrations because the most contaminated areas were in less saline waters. Alternatively, the inverse relationship with salinity may reflect the inclusion of female crabs in the higher-salinity waters. Female crabs are reported to spend part of the year spawning in the Gulf of Mexico [24] and thus would be less exposed to PCDD/F contamination. However, these relationships explained little of the variance in tissue concentrations.

The SEMs for OCDD in BC (Fig. 6F) explained only 23% of the variability in tissue concentrations; only two seasonal effects, air temperature and day number, exhibited statistically significant relationships with tissue concentrations. No predictor variables exhibited statistically significant relationships with OCDF levels in BC tissues.

CONCLUSIONS

As predicted by EqP theory, the important influences of chemical properties, chemical concentrations in water and sediment, and tissue lipid content were apparent in the bioaccumulation of PCDD/F congeners in HH and BC of the HSC system. However, a large percentage of the variation in bioaccumulation could not be explained by EqP theory, indicating the great complexity of the system. This was particularly true for the penta- to octa-chlorinated congeners. Bioaccumulation factors and BSAFs declined with degree of chlorination for PCDD congeners, which may reflect steric constraints on membrane permeation, low dietary uptake efficiency, or reduced bioavailability due to strong sorption to bed and suspended sediment and colloidal phases. An apparent reduction in BAFs and BSAFs with increased concentrations in the sediment and water phases may be explained by the variable exposure of mobile organisms along wide-ranging spatial and temporal gradients of chemical contamination.

For both BC and HH, tissue concentrations appeared to be related more closely to sediment than water concentrations. This implies that sediments are the more important route of exposure for PCDD/Fs. Remediation efforts focused on legacy sediment contamination may be most effective in reducing tissue burdens of PCDD/Fs.

Other factors also apparently affect the extent of bioaccumulation in this system. These may include seasonal factors related to the organisms' temperature preferences and reproductive behavior, seasonal recruitment, the mobility of the organisms together with spatially heterogeneous concentrations in sediment and water, and the size and age of individual organisms. It is important to evaluate the risks of contaminant bioaccumulation in light of the temporal and spatial variability of exposure, as well as the mobility and life history of the species. After considering known variables, a large pool of unexplained variance existed in the levels of bioaccumulation, particularly for the more chlorinated congeners. Some of this variation may be explained by analytical uncertainty; however, biotransformation may also be an important factor in controlling the levels of bioaccumulation.

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